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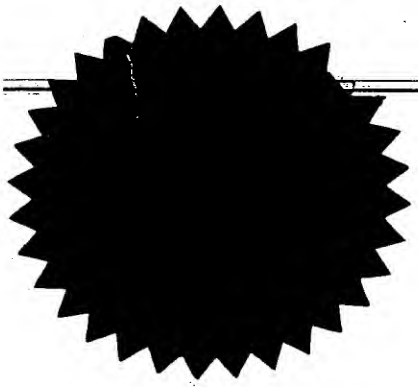

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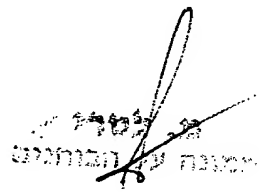

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בקשה לפטנט
Application For Patent

לשימוש הלשכה
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אני, (שם המבקש, מענו ולגבי גוף מאוגדת מקום התאגדותו)
I, (Name and address of applicant, and in case of body corporate-place of incorporation)

רמות רשות אוניברסיטאית למחקר שימושי ולפיתוח תעשייתי בע"מ , חברה ישראלית, מרח' חיים לבנון 32, רמת-אביב, 69975, ישראל

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רצפי DNA, חלבונים המקודדים על ידיהם תרופות ושיטות זיהוי המשתמשים בהם

(באנגלית)
(English)

DNA sequences, proteins coded by them, medicaments and detection methods using same

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המען למסירת מסמכים בישראל
Address for Service in Israel

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חתימת המבקש
Signature of Applicant

For the Applicants,
REINHOLD COHN AND PARTNERS
By : —

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רצפי DNA, חלבונים המקודדים על ידיהם תרופות ושיטות זיהוי המשתמשים בהם

DNA sequences, proteins coded by them, medicaments and detection methods using same

רמות רשות אוניברסיטאית למחקר שימושי ולפיתוח תעשייתי בע"מ

Ramot University Authority for Applied Research & Industrial Development Ltd.

C.110699

FIELD OF THE INVENTION

This invention relates to novel DNA sequences, amino acid sequences coded by them, detection method using said DNA sequences and pharmaceutical composition.

BACKGROUND OF THE INVENTION

Iron is known to be an essential element of the makeup of every living organism, but may also become toxic at physiological pH values by virtue of its tending to oxidize, hydrolyze and precipitate as insoluble ferric oxide polymers. The protein ferritin, found in all living cells, is the body's means for ensuring that iron toxicity does not occur. Ferritin functions by storing iron in the cells in a soluble and readily available form. The iron stored in cells may then be mobilized whenever needed by the body, for example for erythropoiesis.

The name *"ferritin"* actually encompasses a number of individual isomeric forms which are characteristic of different tissue types. Each isoferritin has 24 subunits of two distinct types, being light subunits (L) and heavy subunits (H). These subunits differ in molecular weight, the light subunit being about 18 kDa, and the heavy subunit about 19-21 kDa. The isoferritins extracted from different tissues or organs typically exhibit different isoelectric points, with the isoelectric focusing pattern of human

tisues forming a continuous spectrum; those tissues associated with high iron storage have ferritins at the basic end of the spectrum (e.g. spleen and liver), while iron poor tissues, (e.g. heart and placenta) and malignant cells have acidic ferritins. (Drysdale, *Ciba Found. Symp.*, **51**:41, 1977). The difference in isoelectric point appears to be related to the different distribution of light and heavy subunits in each type. Specifically, heavy subunit-rich ferritins are relatively acidic, and light chain rich ferritins are relatively basic (Cosell, *et al.*, in *Ferritins and Isoferritins as Biochemical Markers*, p. 49-65, 1984, Elsevier). Current studies indicate that the H and L subunits are encoded by a complex group of genes.

A specific type of acidic isoferritin has been shown to be characteristic of neoplastic cells and placental cells (Drysdale and Singer, *Cancer Res.*, **44**:3352, 1974). This protein is also known as oncofetal ferritin or placcental isoferritin (PLF). Human placental ferritin has been shown to be composed predominantly of a single subunit type comigrating with a liver ferritin standard on SDS-PAGE (Brown *et al., Biochem. J.*, **182**:763, 1979). However, an immunoradiometric assay performed with anti-human spleen ferritin has shown tissue specific antigenicity for PLF (Brown *et al., supra*). A three subunit structure has been revealed for PLF (Moroz *et al., G.I. Pat. Clin.*, 1:17-23, 1986). In addition to the L and H subunits characteristic of all ferritins, there is also a high molecular weight (43 kDa) subunit which appears to be unique for human placenta, and thus provides a potential site for identification of the placental isoferritin molecule as distinguished from any other type of ferritin.

Various ferritin isoforms have been isolated from normal and malignant tissues, the most acidic ones predominating a tumor and fetal tissues (Drysdale, 1976, Ciba Found. Symp. **51**:41; Arosio *et al., J. Biol. Chem.*, **253**:4451, 1978). It has been suggested that the assay of acidic isoferritin in the serum may be of value in the diagnosis of malignance

(Hazard *et al., Nature*, **265**:755, 1977). Elevated concentrations of serum ferritin were found in patients suffering from a variety of malignant diseases, including acute lymphocytic leukemia (ALL) (Matzner *et al., Am. J. Hematol.*, **9**:13, 1980), hepatoma (Giannoulis, *Digestion*, **30**:236, 1976) and recently Hodgkin's disease (Bezwoda *et al., Scand. J. Haematol.*, **35**:505, 1985). In assays based on antibodies against HeLa cell ferritin, Hazard and Drysdale found higher concentrations of ferritin in sera from patients with various tumors than in the same sera assayed by antibodies directed against normal liver ferritin (Hazard, *et al., supra.*). Others have failed to demonstrate a consistent pattern of isoferritins in tumor tissues (Cragg *et al., Br. J. Cancer*, **35**:635, 1977; Halliday, *et al., Cancer Res.*, **36**:4486, 1976) or in sera obtained from patients with tumors (Jones, *et al., Clin. Chim. Acta.*, **85**:81, 1978; Jones, *et al., Clin. Chim. Acta.*, **106**:203, 1980).

Although publications concerning the existence of oncofetal ferritin or placental isoferritins have been evident at least since the year 1976, up until today the sequence of this protein and the gene encoding therefor were not known. This is probably due to the fact that the protein itself is hydrophobic and almost devoid of iron and as a consequence extremely sticky and not capable of sedimentation even by high speed centrifugation thus hindering its isolation and purification. In addition, the sequence of the gene coding for oncofetal ferritin could not be found in regular cDNA libraries, probably due to the fact that its expression in these libraries is extremely low. The protein is secreted only by the placenta during pregnancy or by cancer cells in malignant diseases such as lymphoproliferative disorders, breast cancer and in HIV infection.

Breast cancer is a malignant disease effecting different populations at a rate of one to every 9-13 of women. Early diagnosis of breast cancer is known to considerably improve the prognosis of the patient. Diagnosis of breast cancer is based today mainly on imagining techniques, such as mammographs

verified at times by biopsies. Blood-based assays of breast cancer have been reported in the literature, for example, biomarkers such as CA 15.3, (Daly, L. *et al., Comparison of a novel assay for breast cancer mucinto and CA 54 15.3 carcinoembryonic antigen, J. Clin. Oncol.,* **10**:1057-65); the CA 549(2) marker (Dermers, I.M., *et al.,* CA 549: a new tumor marker for patients with advanced breast cancer *J. Clin. Lab. Anal.*, **2**: 168-73, 1988); and the marker CA M29 CEA (Duistrian, A.M., *et al., Evaluation of CA M26, CA M29, CA 15.3 and CEA as circulating tumor markers in breast cancer patients, Tumor Biol.*, **12**:82-90, 1991). However these assays, reported in the scientific community have not gained, to date, clinical significance (Werner, M., *et al., Clinical utility and validation of emerging biochemical markers for mammary adenocarcinoma, Clin. Chem.,* **39/11**(B):2386-96, 1993).

U.S. 4,882,270 discloses an assay for the detection of breast cancer based on determination of oncofetal ferritin. The assay is based on binding of the oncofetal ferritin to specific monoclonal antibodies.

Pathological pregnancy is a term commonly used to describe a multitude of symptoms which create difficulties in carrying a child to term and include spontaneous abortion and miscarriage, premature contractions, toxemia, premature delivery. U.S. 4,954,434 discloses the fact that low levels or absence of PLF in pregnant women can serve as a marker for potentially high risk pregnancy. Detection of this state is again achieved by monoclonal antibodies which has PLF specificity. This patent also concerns treatment and prevention of actual and potentially pathological pregnancy by the administration of this protein. However, since the sequence of the protein was not known at the date of the patents, the treatment suggested involved administration of partially purified protein and not of recombinant pure proteins.

U.S. Patents 5,571,678, 5,120,640 and 5,283,177 are all directed to methods for assaying the presence and evaluating the prognosis of acquired

immunodeficiency associated with HIV induction, by determining levels of placental isoferritin by monoclonal antibodies.

All the above detection methods concern antibody-based assays. While such assays are known to be useful in conditions where the level of the protein to be detected is quite high, they are notorious for eliciting a false-negative answer where the protein level is low. Against this, assays based on amplification of mRNA (RT-PCR) are much more sensitive and can detect even minute expression of mRNA. Thus there is need today for a RT-PCR method for detection of oncofetal ferritin for detection of breast cancer and for diagnosis of high risk pregnancies at its early stage.

Furthermore, it would have been desired to provide pure oncofetal ferritin protein prepared by recombinant processes for therapeutic and vaccination purposes.

SUMMARY OF THE INVENTION

The present invention is based on the finding of the sequence of the oncofetal ferritin gene and of the oncofetal ferritin protein. The inventor was the first to discover the full sequence of the gene that codes for the protein termed hereinafter as *"oncofetal ferritin 1 (OFF1) protein"*.

Thus, the present invention concerns a DNA sequence coding for the subunit of the oncofetal ferritin protein termed *"oncofetal ferritin 1"* (OFF1) protein selected from the group consisting of:

(i) a DNA sequence as depicted in Fig. 1;

(ii) a DNA sequence as depicted in Fig. 4;

(iii) a DNA sequence which codes for the same amino acid sequences encoded by the sequence of (i) or (ii);

(iv) fragments of any of the sequences of (i) to (iii) that code for a functionally equivalent gene product;

(v) a DNA sequence that has at least 80% homology, as determined by hybridization under stringent conditions, to any one of the sequences of (i) to (iv) and code for a physiologically active protein.

(vi) a DNA sequence that hybridizes to the sequences of (i) or (iv), or to the clone deposited with the ATCC, under highly stringent conditions, e.g., hybridization to filter-bound DNA in 0.5M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C, (Ausubel F.M. *et al.* eds., 1989, *Current Protocols in Molecular Biology,* Vol. I, *Green Publishing Assoc., Inc. and John Wiley & Sons., Inc. New* York *at p. 2.10.3),* which can either be used as a probe for OFF1, or which encodes functionally equivalent gene product; and

(vii) a DNA sequence that hybridizes to the sequences of (i) to (iv), or to the clone deposited with the ATCC, under moderately stringent conditions, e.g., washing in 0.2xSCC/0.1% SDS at 42°C (Ausubel et el., 1989, supra), yet which still encodes a functionally equivalent gene product.

The sequence of Fig. 1 was isolated from a cDNA library obtained from breast cancer patients while the DNA sequence of Fig. 4 was obtained when using PCR amplification where the sequence of Fig. 1 was used as a template. The two sequences differ only in the 5′ non-coding region which include 2 single nucleotide substitutions as well as a single base insertion and one deletion.

The DNA sequences of the invention also include DNA sequences which code for the same amino acid sequences as those of Figs. 1 or 4. It is known that due to the degenerative nature of the genetic code, a large number of alternative DNA sequences, may code for the same proteins. Thus all

sequences which code for the same amino acid sequences are encompassed by the scope of the invention.

The present invention further concerns DNA sequences having at least 80% homology either to the DNA sequence of Fig. 1 or 4, or to the DNA sequences coding the same types of amino acid sequences, the homology determined by hybridization under stringent conditions. Examples of highly stringent conditions are given in **(vi)** above and of moderately stringent conditions are given in **(vii)** above. The artesian will appreciate the fact that there exists a large number of sequences capable of hybridization under such conditions, some of which code for more physiologically active OFF1 proteins than others. Those sequences which fall under the scope of the invention, are those which code for a functionally equivalent gene product, as will be explained hereinbelow.

The present invention also encompasses DNA sequences that hybridize to the sequences of Figs. 1 or 4, or to the cDNA inserts contained in the deposited cells, under highly stringent conditions, as specified above. Such DNAs can be used as probes to detect the OFF-1 gene or mRNAs (e.g. by hybridization or PCR amplification assays). Alternatively DNAs that hybridize under highly stringent or less stringent conditions (specified above), yet which encode a functionally equivalent gene product are also encompassed by the invention.

The present invention further concerns fragments of all the above sequences, which code for an OFF1 protein having functionally equivalent gene product will be explained hereinbelow.

The term *"functionally equivalent gene product"*, refers to an amino acid sequence, which is physiologically active in a manner similar to that of the native OFF1 protein. Such an activity can be tested, for example, by determining the immunosuppressive activity in cell mediated immunity, as well known in the art.

The present invention further concerns expression vectors comprising said DNA sequences, as well as a host cells transfected with such expression vectors. Expression may be obtained in any suitable pro-or eucaryotic expression systems using known methods e.g. as described in Genentech EP 200341.

Suitable expression vectors are DNA sequences encoding OFF1 and operably linked to suitable control sequences capable of effecting the expression of OFF1 in the host. Such control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences that control termination of transcription and translation. For expression of OFF1 in eukaryotic cells, the vector also should include DNA encoding a selection gene.

Vectors include plasmids, viruses (including phages) and integratable DNA fragments, i.e. fragments that are integratable into the host genom by recombination.

Preferred host cells are cells derived from multicellular organisms. In principle, any higher eucaryotic cell culture is workable whether from vertebrate or invertebrate culture. Examples useful in host cell lines are Chinese Hamster Ovary (CHO) cell lines and COS 7 cell lines.

In another embodiment of the present invention, the cells and tissues may be engineered to express an endogenous gene under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional

initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting, including polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, , e.g., inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the host cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes simplex virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin *et al.*; International Application No. PCT/US92/09627 (WO93/09222) by Selden *et al.*; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi *et al.*, each of which is incorporated by reference herein in its entirety.

The present invention of course concerns also a recombinant protein coded by the above DNA sequence. Said recombinant protein is in fact the first recombinant OFF-1 protein produced, and enables production of large amounts of such protein in a pure form for therapeutic and detection purposes as will be explained hereinbelow.

The present invention further concerns anti-sense RNA sequences, which are complementary to the mRNA sequences transcribed from the above DNA sequences, and thus are capable of neutralizing the expression of native OFF-1 gene in cells. The present invention further concerns DNA sequences coding for said anti-sense mRNA, as well as expression vectors comprising said DNA sequences.

The present invention also concerns pharmaceutical compositions of two types.

According to the first aspect of the invention, termed *"the OFF1 activating aspect"* the pharmaceutical compositions of the invention comprise DNA sequences coding for the OFF1 protein, expression vectors comprising said DNA sequences, for expression in specific target cells, or the recombinant OFF1 protein itself. The above agents may be used in the immunization of a subject against diseases which are manifested by abnormally high expression of the OFF1. Examples of such diseases are: cancer, in general, and breast cancer and lymphomas in particular as well as HIV infections.

The pharmaceutical composition according to the activating aspect of the invention may be used, as such, for increasing the level of the OFF1 protein for the treatment of conditions manifested by lower than normal levels of the OFF1 protein. If the level of the OFF1 protein can be raised again to normal level, either by administering to the subject recombinant OFF1 protein, or by transfecting the subject's target cells with an expression vector comprising the DNA coding for the OFF1 protein, then the pathological conditions may be alleviated. Pathological conditions treated by these pharmaceutical compositions are pathological pregnancies manifested by spontaneous abortion and miscarriage, premature contractions, toxemia and premature delivery. In addition, the pharmaceutical compositions may be used to inhibit transplant rejection, for example, specific T-cell mediated immunity like that of a mother against her embryo.

Using the same principal, the pharmaceutical compositions of the invention may also be used for the treatment, alleviation, or prevention or autoimmune diseases, such as: Coeliac disease, Rheumatoid arthritis, Multiple Sclerosis which are T-cell mediated autoimmune diseases.

By another option, the pharmaceutical composition of the activation aspect of the invention may be used to support normal pregnancies, for example, for increasing the chances of success of *in vitro* fertilization (IVF), in both human and non human subjects.

According to another surprising finding it was found that OFF1 can serve as a growth factor for bone marrow progenitor cells. Thus the pharmaceutical composition of the invention may be used to enhance growth of bone marrow progenitor cells, for example, where due to some pathological condition their number decreased. For example in patients treated by mega doses of cytotoxic drugs which kill bone marrow cells such as cancer patients , HIV infected patients and the like. In addition the pharmaceutical compositions of the inventions could be used in patients who

need bone marrow replacements such as those with genetic metabolic defects or autoimmune diseases.

By another alternative the DNA coding for OFF1 can be used in the development of a chimera which will enable further engraftment of organ allografs or xenografts identical with the bone marrow donor.

In addition, a preparation comprising OFF1 can be used *ex vivo* as a growth factor for bone marrow progenitor cells, for example, from bone marrow obtained from a donor prior to implantation.

An example of bone marrow progenitor cells is granulocyte monocyte progenitor cells.

By another aspect of the invention, termed *"the OFF-1 neutralizing aspect"* the pharmaceutical compositions of the invention comprise anti-sense mRNA to the OFF-1, expression vectors comprising DNA sequences coding for said anti-sense mRNA, or antibodies against the OFF-1 protein. The neutralizing aspect of the invention, is intended to lower the levels of OFF-1, where it is abnormally raised as compared to normal tissue, notably for the treatment of cancer, especially breast cancer.

By another option the neutralizing aspect of the invention may be used in order to reduce the normal level of OFF1 which is required to maintain a pregnancy to term and thus cause abortion (Moroz, C., 9th International Congress of Immunology, San Francisco, 1995).

The present invention further concerns a method for the detection of cancer, or for the evaluation of the prognosis of a cancer patient, by determining the level of the OFF1 gene expression in said patients.

U.S. Patent 4,882,270 discloses a method for detecting breast cancer, by using antibodies against isoferritin placental protein.

This method detects said protein in early stages of the cancer only on lymphocytes not in the serum. The protein can be detected in the serum only

at a very late stage of the disease when the tumor has already metastasized. The problem with lymphocyte-based detection, is double fold: first, technical issues concerning detection are quite severe, since there is a requirement to isolate fresh lymphocytes from the blood and assay within hours without an opportunity to retest at a later date. This severe technical problem prohibits the use of this method of detection in widely used screening assays. Second, during progressive stages of cancer, the number of positive lymphocytes decreases dramatically, and thus it is not possible to detect the cancer, using lymphocytes-directed anti-ferritin antibodies. Thus, according to said U.S. patent, it is possible to detect cancer at its early stage, where the number of lymphocytes is high, as well as at the very last stages, where the level of the isoferrin placental protein is so high that it is shed to the serum, but in most stages of the cancer, the low number of positive lymphocytes avoids detection of cancer.

Against this, the detection methods of the present invention, are based on RNA amplification, and are sensitive enough to detect even slightly elevated levels of the OFF1 mRNA present in small amounts of the patient's blood in virtually all stages of cancer . The method of the invention detects the mRNA in circulating cancer cells, whereas the protein is shed from tumors and binds to lymphocytes.

In addition to detection of the presence of cancer, the level of OFF1 protein is also a good indicator of the prognosis of cancer, for example the change in mRNA level after removal of the tumor by surgery or chemotherapy may indicate disease prognosis.

Examples of cancers which can be detected by the methods of the invention are breast cancer, hepotomas, leukemias, lymphomas and embryonal tumors such as neuroblastoma and hepatoblastoma.

In addition, elevated levels of OFF1 expression, are typical of Down Syndrome. In Down Syndrome there are elevations of embryonal proteins like

α-fetoprotein (AFP). Also the Syndrome is associated with decreased immunoreactivity and high incidence of cancer. Thus by detecting high levels of this protein it is possible to determine also Down's disease.

By another embodiment, the present invention concerns a method for the detection of diseases connected with pathological pregnancies, comprising detecting a lower level than normal of the OFF-1 expression. The term *"pathological pregnancies"* groups together a large number of disorders including spontaneous abortion and miscarriage, premature contractions, toxemia, premature delivery.

The detection of the level of the OFF-1 expression, both for determining higher than normal levels (various types of cancer) or lower than normal levels (various types of pathological pregnancies) can be carried out by utilizing reverse transcriptase polymerized chain reaction (RT-PCR). This method, considerably amplifies the OFF-1 mRNA present in the blood enabling its detection, even in minute levels.

The present invention further concerns a method for the isolation of the cDNA of the invention as specified in Fig. 1 or 4 as will be appreciated in the *"Detailed Description"* section of the specification.

The present invention further concerns primers for use in the above isolation method. These primers may also be used in RT-PCR, for the detection purposes of the invention. The primers are selected from the group consisting of:

5′ GGT GGC GAC GAC TCC TGG AGC CCG 3′
5′ TTG ACA CCA GAC CAA CTG GTA ATG 3′
5′ GAC CGC GAT GAT GTG GCT TTG AAG AAC 3′
5′ GAT AGG ATC TTT AGC GAC AGC CGA 3′
5′ ATG GCG GCC TCT GAG TCC TGG TGG 3′
5′ CGG GCT GAA TGC AAT GGA GTG TGC 3′
5′ GAC CCC CAT TTG TGT GAC 3′
5′ CGA CGA CTC CTG GAG CCC G 3′
5′ Biotin-TTG ACA CCA GAC CAA CTC GTA ATG 3′
5′ AGC CGA CAG CGA TTT CTA GGA TAG 3′
5′ GTT CTT CAA AGC CAC ATC ATC GCG GTC 3′
5′ GCT TTC ATT ATC ACT GTC TCC CAG GGT G 3′
5′ CAG ACG TTC TTC GCC GAG AGT CGT 3′
5′ CAG ACG TTC TTC GCC GAG AGT CGT CGG 3′
5′ CAT TTC GGG GAT TCG GGG GA 3′
5′ GGG GGA CGG AAC CCG GCG CT 3′
5′ CCC TCT ACA CTT ATC ATC TTC 3′
5′ CTA TCC TAG AAA TCG CTG TCG GCT 3′
5′ GTC ACT ACT GGA ATT CCC TTC TCC 3′
5′ GGA GAA GGG AAT TCC AGT AGT GAC 3′
5′ GGA AAT CGC TGT CGC CTA ACC 3′
5′ GGT TAG GCG ACA GCG ATT TCC 3′
5′ GGC CAC GCG TCG ACT AGT AC 3′
5′ GTA ATG CAC ACTCCA TTG GC 3′
5′ GTA ATG CAC ACT CCA TTG 3′
5′ GCG CTC AGC TGG AAT TCC 3′
5′ GGA ATT CCA GCT GAG CGC 3′
5′ GTG GGA TCC CCA TGA CGA CCG CGT CCA CC 3′
5′ GAC TCG AGT TAA GCC GAC AGC GAT TTC 3′
5′ GAC TCG AGT CAG GGT GAC CGA AAA ATC AG 3′
5′ CCC GCT CGA GTC AGG GTG ACC GAA AAA TCA G 3′

BRIEF DESCRIPTION OF THE DRAWINGS:

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 shows the nucleic acid sequence of clone T16 isolated from T47D breast cancer cDNA library. Initiation and termination codons of the open reading frame are indicated by dark bars;

Fig. 2A shows a comparison of the nucleic acid sequences (upper sequence) of clone 4.7 isolated from a placenta cDNA library exhibiting normal human FTH, and the sequences (lower sequence) of clone T16 isolated from human breast cancer T47D cDNA library. Initiation and termination codons of the open reading frame are marked by dark boxes;

Fig. 2B shows in a schematic representation the comparison of the two sequences shown in Fig. 2A. Differences in nucleic acid sequences are represented by the shaded areas;

Fig. 3 shows a comparison of sequence homology between cDNA clone T16 and human mitochondrial cytochrone oxidase I DNA;

Fig. 4 shows a comparison of nucleic acid sequences between placental cDNA obtained by PCR amplification using T16 specific primers (upper sequence) and T16 cDNA sequence obtained from the T16 cDNA clone (lower sequence). Identical nucleic acid sequences are indicated by a dotted line. Initiation and termination codons are indicated by a dark bar;

Fig. 5 shows the nucleic acid sequence and deduced amino acid sequence of the cDNA of OFF1;

Fig. 6A shows the relative expression of FTH in mRNA and OFF1 RNA among total mRNA isolated from different tissues and optimized with β-actin expression;

Fig. 6B shows the relative expression of FTH mRNA hybridized with 32^{P}Pst1 3′ fragment of liver FTH cDNA (530 bp), from either normal HBL human lactating breast or from cancer cell lines (MCF-7 and T47D);

Fig. 7 shows the sequence of clone T16. primers used for PCR are indicated in the above sequence;

Fig. 8 shows the restriction enzyme map sequence of clone T16;

Fig. 9A shows SDS-PAGE (12%) of total cell lysates (lane 2) 10 μl from *E.coli* containing the vector pGEX alone or the pGEX constructed to contain C48 fragment (lane 4) or total constructs containing full length OFF1 (lane 6). The recombinant proteins are marked by arrows;

Fig. 9B shows the same as Fig. 9A but reactive with CMH-9 in antibodies (lanes 4 and 6 indicating presence of protein);

Fig. 10 shows the effect of the protein of the invention on CFU-GM colony formation obtained from three healthy donors; and

Fig. 11 shows the effect of the protein of the invention and its combination with GM-CSF or CMH9 Moab on CFU-GM colony formation.

DETAILED DESCRIPTION OF THE INVENTION

EXPERIMENTAL PROCEDURES

Example 1 Cloning of OFF1 cDNA

λgt11 cDNA libraries were prepared using total poly A^{+}RNA from human breast cancer cell line T47D or from human placenta. Both libraries were randomly primed from total poly $(A)^{+}$RNA. EcoRI linkers were attached to the cDNAs which were inserted into the EcoRI site of the bacteriophage λgt11.

About 10^{6} plaque forming units were screened by using human liver ferritin (FTH) cDNA provided by R. Cortese (Constanzo *et al., EMBO*, 3:23-27, 1984). Plaque hybridization was carried out according to Berton, W.D. and Davis R.W., (*Screening λgt recombinant clones by hybridization*

to single plaques in-site, Science, **196**:180-182 (1997)). In brief, plaque hybridization was carried out at 42°C, 5xSSC and subsequent washing 3 times with 2xSSC 0.1% SDS at room temperature and twice with 1xSSC, 0.1% SDS at 68°C.

Clones were isolated that gave hybridization signals after two rounds of screening. PCR amplification was performed on clones using one primer from the λgt11 vector (λgt11 F-1060 or λgt11 R-1061, Table 1) and one FTH gene-specific primer (either 17R to amplify toward the 5′ end of 17F to amplify toward the 3′ end; as indicated in Table 1) as described above, PCR amplification was performed for 30 cycles under standard conditions. PCR products from the clones derived from both the 5′ and the 3′ end of the cDNA clone were selected according to their size, so that their sequence would produce a contig of maximum length. PCR products were purified by Qiagen PCR purification columns according to the manufacturer's instructions and were sequenced using standard protocols for the ABI373 or 377 primer mated sequencer with the 1060 and 1061 primers (Table 1) and specific primers until the full sequence was determined.

Table 1

List of Primers

Name	#MR	Sequence		
1060F	24	5′ GGT GGC GAC GAC TCC TGG AGC CCG 3′	75%	
1061R	24	5′ TTG ACA CCA GAC CAA CTG GTA ATG 3′	45.80%	
17F	27	5′ GAC CGC GAT GAT GTG GCT TTG AAG AAC 3′	52%	27618
X1.1F	24	5′ GAT AGG ATC TTT AGC GAC AGC CGA 3′	50%	24880
X.1.1R	24	5′ ATG GCG GCC TCT GAG TCC TGG TGG 3′	67%	
2.1F	24	5′ CGG GCT GAA TGC AAT GGA GTG TGC 3′	58%	
3.4F	18	5′ GAC CCC CAT TTG TGT GAC 3′	55.50%	
1060F/S	19	5′ CGA CGA CTC CTG GAG CCC G 3′	73.70%	
1061r/Bio	24	5′ Biotin-TTG ACA CCA GAC CAA CTC GTA ATG 3′	45.80%	
16X.1R	24	5′ AGC CGA CAG CGA TTT CTA GGA TAG 3′	50%	24879
17R	27	5′ GTT CTT CAA AGC CAC ATC ATC GCG GTC 3′	52%	27385
3′COD R	28	5′ GCT TTC ATT ATC ACT GTC TCC CAG GGT G 3′	50%	28313
5′ NCF	24	5′ CAG ACG TTC TTC GCC GAG AGT CGT 3′	58%	24870
4869	27	5′ CAG ACG TTC TTC GCC GAG AGT CGT CGG 3′	63%	
NFG	20	5′ CAT TTC GGG GAT TCG GGG GA 3′	60%	
NFGP-2	20	5′ GGG GGA CGG AAC CCG GCG CT 3′	80%	201880
767-F	21	5′ CCC TCT ACA CTT ATC ATC TTC 3′	43%	211616
16-F	24	5′ CTA TCC TAG AAA TCG CTG TCG GCT 3′	50%	241173
ECO-F	24	5′ GTC ACT ACT GGA ATT CCC TTC TCC 3′	50%	24960
ECO-R	24	5′ GGA GAA GGG AAT TCC AGT AGT GAC 3′	50%	24961
SPF	21	5′ GGA AAT CGC TGT CGC CTA ACC 3′	57%	211667
SPR	21	5′ GGT TAG GCG ACA GCG ATT TCC 3′	57%	211668
AUAP	20	5′ GGC CAC GCG TCG ACT AGT AC 3′	65%	202738
NC-F	20	5′ GTA ATG CAC ACTCCA TTG GC 3′	50%	203814
SNC-F	18	5′ GTA ATG CAC ACT CCA TTG 3′	44%	181897
BNC-F	18	5′ GCG CTC AGC TGG AAT TCC 3′	55.50%	181898
BNC-R	18	5′ GGA ATT CCA GCT GAG CGC 3′	61.10%	181905
pGEX-F	29	5′ GTG GGA TCC CCA TGA CGA CCG CGT CCA CC3′	67%	29391
pGEX-R1	27	5′ GAC TCG AGT TAA GCC GAC AGC GAT TTC 3′	51.85%	27578
pGEX-R2	29	5′ GAC TCG AGT CAG GGT GAC CGA AAA ATC AG 3′	51.70%	29396
pGEX-R3	31	5′ CCCGCTCGAGTCAGGGTGACCGAAAAATCAG 3′	58%	31277

Example 2 Sequence analysis

The cDNA sequences and its deduced protein sequence were used to search the complete combined Gene Bank/EMBL database and the complete Swiss Prot database with BLAST and FASTA programs, respectively.

Example 3 RNA preparation and northern blot analysis

Total RNA was extracted from cells by guanidinium isothiocyanate solubilization and prepared by phenol-chloroform extraction. PolyA$^+$ RNA was purified using 2 rounds of oligo(dT) chromatography. Total RNA (25 μg) was separated by electrophoresis through 1% agarose, 2.2 M formaldehyde gels, transferred to nylon membranes (Hybond-N, Amersham, Arlington Heights, IL) and fixed by baking at 80°C for 2 hrs. The probes used for hybridization were:

(1) PstI 3′ fragment of FTH cDNA (530 bp);

(2) T16 specific SPF-16R PCR cDNA product.

After hybridizations with ^{32}P-labeled probes (10^6 cpm ml) 42°C, 50% formamide 5xSSC, blots were washed in 2xSSC, 0.1% SDS at 25°C, followed by 0.1xSSC, 0.1% SDS at 55°C.

Human tissues mRNA was purchased from Clontech (Cat. # 7760-1) and hybridized according to the manufacturer's instructions.

Example 4 Detection of OFF1 transcripts by RT-PCR in peripheral blood

1. Preparation of blood derivatives

Tube A. To whole blood (0.5 ml) 1 ml red blood cell lysis buffer (Boehringer) was added at room temperature and mixed by inversion (without vortex). The preparation was then stored for 10 mins. at room temperature with agitation, and

centrifuged at 2,500 rpm for 5 mins. at room temperature. The pellet was then washed with PBS.

Tube B 4.5 ml whole blood were centrifuged for 5 mins. at room temperature, at 1,030 g. In order to buffy coat, about 0.5 ml lysis buffer 1 ml was added for 10 mins. at room temperature. The mixture was placed on a shaker, and then centrifuged at 2,500 rpm for 5 mins. at room temperature. The pellet was finally washed with PBS.

Tube C To 5 ml of whole blood 10 ml of PBS were added. Mononuclear bells were isolated by density centrifugation on Ficoll hypaque (5 ml), and spun at 450 g for 25 mins. at room temperature. The pellet was washed with PBS (resulting in about $5x10^6$ lymphocytes).

II. Isolation of RNA (according to Tri-reagent protocols supplied by the manufacturer)

1. Cells were lysed with Tri Reagent; To Tube A – 0.5 ml were added, to Tubes B and C 1 ml was added by repetitive pipetting.
2. The preparation was stored for 5 mins. at room temperature, and then 0.2 ml chloroform per 1 ml of Tri Reagent were added and vortexed for 5 secs. The resulting preparation was stored for 2-15 mins. at room temperature and centrifuged 12,000 g 15 mins. at 4°C.
3. The aqueous phase was transferred to a fresh tube, and precipitated by 0.5 ml isopropanol per 1 ml TRI Reagent on ice for 5-10 mins. (or at room temperature) and centrifuged 12,000 g at 4°C.

4. The supernatant was removed and the RNA pellet was washed with 75% ethanol by vortexing and centrifugation.

The RNA pellet was air dried and then dissolved in 20 µl DEPC- water, RNA has O.D. at 260/280 = 1.6-1-9 yield = 2.5 µg (for Tubes B and C).

III. RT-PCR

1 µg RNA (or 14 µl whole blood RNA of Tube A) was placed in total volume of up to 15 µl in DEPC water, heated at 70°C for 10 mins. and cooled immediately on ice. Then it was spun briefly and 10 µl of mix was added a follows: 5 µl M-MLV RT 5xReaction buffer; 1 µl dNTPs (12.5 µl); 1 µl Recombinant Rnasin Ribonuclease (20 u); 2 µl DEPC water; 1 µl M-NLV Reverse transcriptase (RT) (200 units).

The mixture was incubated for 60 mins. at 37°C followed by 10 mins. at 70°C to stop the reaction.

PCR

For PCR the following reagents were used: 1 µl cDNA (or 0.5 µl cDNA for PCR of normal ferritin (FTH) H (Chain); 5 µl 10x reaction buffer for DNA polimerase; 1 µl forward primer (~ 10 pmole); 1 µl reverse primer (~ 10 pmole); 1 µl dNTPs (12.5 mM), 0.5 u Taq polymerase from Appligen (0.1 µl); Takara 0.2 gel DDW water up to 50 µl.

The PCR program (cycles) was as follows:

1) 94° - 2′	2) 50° - 2′	3) 72° - 1′
4) 94° - 1′	5) 50° - 2′	6) 72° - 3′
7) 29 times to 4	8) 94° - 1′	9) 50° - 2′
10) 72° - 10′	11) 4°.	

PCR products were identified by electrophoresis on 1% agarose gel and 0.10 μg ethidiumbromide.

Since T16 transcripts do not yield bands after the first PCR, in order to amplify the results the initial PCR is followed by nesting PCR, using 1 μl of PCR 1 diluted 1:100 in the above PCR program.

As an example, the following primers were used for nesting PCR

PCR1: XIF → 16R

nesting PCR 2: 17F → SPR

For normal ferritin: 17F → 3′R yields a visible product.

No nesting is required.

RESULTS

Example 5 Isolation of cDNA clones

Five cDNA clones were isolated from T47D breast cancer cells cDNA library (T) and 15 cDNA clones were isolated from placenta cDNA library (P). The two clones with the largest inserts (1 KB) were T16 from T47D breast cancer and p4, 7 from placenta.

The sequence of the full length cDNA (0.9 KB) from clone T16 revealed a sequence of 890 bp; 109 bp in the 5′ UTR 495 bp (165 aa) in the coding region and 286 bp in the 3′ UTR. Full sequences are shown in Fig. 1.

The nucleic acid sequence of cDNA from clone p4, 7 revealed a sequence of 890 bp is completely homologous to the known FTH sequence (as compared in Fig. 2) and represents a normal ferritin heavy chain (Cohen *et al.*, 1996, Drysdale 1988). Partial homology was found between clone p4, 7; and clone T16 (Fig. 2A, 2B). The homologous sequences were clone 4, 7 139-511 and clone T16 87-460. The later included 22 bp in the 5′ UTR

followed by 351 bp (117 aa) in the above coding region. No further homology was found between the above two clones. As can be seen in Fig. 2A and B, homology is indicated by a broken line.

Example 6 Comparison to Gene Bank data

Comparison of the new sequence of T16 to sequences in the Gene Bank and EMBL to database show only a segment from 463-671 bp matching a segment of human mitochondria cytochrome oxidase I (COI) 6486-6694 bp (Fig. 3). However, the predicted 48 amino acids of T16 did not match the CO I sequence. There was no further homology found in the Gene Bank for T16; 671-890 bp or I-87 bp.

Example 7 PCR amplification of a T16 compatible placental cDNA

A placenta λgt11 cDNA library was used to amplify by PCR a T16 compatible cDNA from placenta. This was performed using the T16 specific primers i.e. λgt11 F (1060) or λgt11 R (1061) and primer 16R for the 5′ end and primer SPF for the 3′ end (Table 1; Fig. 7) schematically as shown below:

1060 5′ _____ 16 3′

5′ SPF _____ 1061 3′

The PCR products obtained from the placenta cDNA library using the above T16 (presented in Fig. 4). There was only a small difference in the 5′ non coding region which included 2 single nucleotide substitutions, as well as a single base insertion and one deletion (Fig. 4).

Verification of the sequence was carried out on cDNA obtained from RNA by reverse transcriptase and PCR amplification (RT-PCR). The RNA

was isolated from human T47D breast cancer and HBL 100 breast epithelial cell lines, and from human peripheral blood lyphocytes ((PBL), non activated and from concanavalin activated PBL.

RNA (5 μg) from the different cells was used to prepare cDNA by reverse transcriptase using random primers. T16 cDNA as amplified by PCR using the following primers:

5′ BNCF and 3′ 16R (Table 1; Fig. 7), followed by nesting with the primers 5′ BNCF-3′ 17R and 5′ 17F-3′ 16R (Table 1; Fig. 7).

5′ BNCF _____ 16R 3′ **1st PCR**

5′ BNCF ---------- 17R 3′ **nested PCR**

5′ 17F _____ 16R 3′ **nested PCR**

Nesting was necessary since the first PCR amplification did not yield a visible PCR product.

The sequences obtained from the isolated cDNA products from the above cell sources revealed that all of them including T47D breast cancer had a sequence identical with the sequence obtained from the T16 homologous cDNA from placenta (Fig. 4), i.e. it included the substitution insertion and deletion in the non-coding region.

These results suggest that the sequence differences in the T16 cDNA clone isolated from the T47D cDNA library were a mistake occurring in the formation of the library. The final nucleotide sequence of T16 and the deduced amino-acid sequence is presented in Fig. 5.

Example 8 Expression of T16 in various tissues

The expression of T16 gene in a variety of human tissues, including breast epithelial and breast cancer cell lines were analyzed by northern blotting, according to state of the art procedure and the results are shown in Fig. 6A and 6B.

The northern blot revealed a 0.9 kb transcript in all the cells tested.

However, as seen in Fig. 6A, 6B, the relative expression of T16 mRNA and FTH revealed that there was over-expression of T16 mRNA only in breast cancer cells indicating that the T16 can be used as a marker for breast cancer.

The relative amount of each tissue mRNA indicates that all the peaks on the blot for a single probe FTH (normal H chain) or T16 (OFF1) amount to 100% and therefore each mRNA bar represents a ratio relative to the other tissues. In order to ensure that the amount of RNA loaded into the gel is equal, a household gene i.e. actin was probed. The numbers are corrected according to ratio of actin. On the same blot therefore if both mRNA (FTH and T16 (OFF1)) are expressed similarly (if one is low/or high the other is also low/or high) then there is no difference in expression between the two mRNAs. However, only in breast cancer there is evidence that T16 (OFF1) is high as compared with FTH but not in HBL cells which is a cell line derived from normal lactating breast. These results clearly indicate that the method of detecting OFF1 is valid for differentiating between breast cancer and cells derived from normal lactating breast.

Example 9 Preparation of OFF1 – Fusion Protein

Materials and Methods

Construction of *E.coli* Strains Expressing Glutathione S-Transferase (GST) and OFF1 Fusion Proteins

The expression vector (pGEX-5X-1) used for gene fusion construction was the GST Gene Fusion System (Pharmacia). The OFF1 coding region (designated as *"FL"*, full-length) of about 0.5 kb was prepared by PCR with the following 5′ end primer:

5′ GT<u>GGGATCC</u>CCATGACGACCGCGTCCA, (BamHI) in order to add a Bam HI site 1 base upstream from the start codon ATG and with the 3′ end primer

5′ CCCG <u>CTCGAG</u> TCA GGG TGA CCG AAA AAT CAG 3′ (XhoI) in order to add an XhoI site after the stop codon TAA using the PCR kit (Perkin-Elmer/Centus).

A deletion construct for encoding the unique C-terminal 48 aa, designated as *"C48"*, of the OFF1 was also prepared from *"FL"* OFF1 PCR product, by cleavage with restriction enzymes 5′ ECORI and 3′ XhoI. The PCR program was as follows (cycles):

1. 94° - 2 min.
2. 94° - 1 min.
3. 50° - 2 min.
4. 72° - 3 min.
5. followed by 28 times
6. 94° - 1 min.
7. 50° - 2 min.
8. 72° - 10 min.

The PCR products were gel-purified and then ligated into the pGEX 5X-1 plasmid at 5′ BamHI and 3′ XhoI sites. The resultant recombinant

plasmids canproduce fusi8on proteins in which the N-terminus is the GST and the C-terminus is the OFF1, or c48 of OFF1. *E.coli* strain BL-21 was transformed with the vector alone or the two recombinant plasmid DNA to produce pGST cells, pGST-FL cells, pGST-C48 cells following the standard protocol (Maniatis Sambrook J. Fritch EF & Maniatis T., Molecular Cloning: A Laboratory Manual (1989) (Cold Spring Harbor Lab. Press Plainview, N.Y. Snd Ed.)).

Growth of *E.coli* Cells and Expression of OFF1 Fusion Protein in *E.coli*

Wild-type and transformed *E.coli* cells were grown in Luria-Bertani (LB) broth containing 100 μg/ml of ampicillin at 37°C overnight. The overnight cultures were diluted 1000-fold using fresh LB broth plus ampicillin, and incubation continued at 37°C. For growth curve determination, samples were taken every 30 mins. to measure the optical density at 600 nm. To test the induction conditions for fusion protein expression, the diluted overnight cultures were grown at 37°C until mid-long phase (3-4 h, or OD_{600}= ≈0.6). Isopropyl β-D-thiogalactopyranoside (IPTG) was then added to a final concentration of 1 mM, and incubation was continued at 37°C for 4 hours. After IPTG induction, the cultures were harvested and cell pellets were obtained by centrifugation. Pellets were resuspended in 100 μl of Laemmli sample buffer. Thirty micrograms of protein samples, determined by the Bradford assay, was subjected to SDS/PAGE.

SDS/PAGE and Western Blot Analysis

One-dimensioned SDS/PAGE was performed according to Laemmli using 12.5% (wt/vol) polyacrylamide gels. For immunoblotting, proteins were transferred from polyacrylamide gels to immobilon polyvinylidene difluoride membranes (Millipore) with Tris/glycine electroblotting buffer

according to Towbin *et al.*[29]. Protein bands cross-reacting with CM-H-9 monoclonal antibody (MoAb) were identified by reaction with horseradish peroxidase conjugated with goat anti-mouse IgG (Bio-Rad). The conditions of immunoreactions were according to the manufacturer's specification (DAKO).

Method for preparation of OFF1 recombinant protein

Day 1:

- Inoculate a single colony of transformed bacteria into 50 ml LB-Borth (with the appropriate antibiotics) and grow overnight.

Day 2:

- Add the 50 ml culture to 1000 ml LB-Borth (with the appropriate antibiotics) and grow for 2 hrs at 37°C (around 0.8 O.D.).
- Add this culture 100 µl of IPTG (stock solution of 1M) and continue growth for 4 hrs at 37°C for 5 hrs at 30°C.
- Centrifuge for 20 mins. at 4°C, 4000 rpm with rotor GS-3.
- Discard the supernatant and suspend in 20 ml ice cold PBS+0.1% Triton-X100.
- Sonicate 90/7 for 10 sec, three times on ice (in 50 ml tube)
- ~~Spin 2300 rpm at 4°C for 10 min.~~
- Collect supernatant in 50 ml Falcon tube
- Add 1 ml of Glutathion-Sepharose 4B Beads (stock 50% beads in PBS).
- Incubate for 5-30 min. at RT or overnight at 4°C with rolling.
- Collect the beads by centrifuging 1 min, 2000 rpm.
- Wash the beads 3 times with ice cold PBS.
- Elute protein with 1 ml of 50 mM Tris-HCl pH 8 containing 15 mM Glutathione and 10 mM DTT.

- Dialyze (twice) against PBS containing 30% Glycerol.
- Store aliquots in -20°C.

The results are shown in Fig. 9A and 9B and clearly indicate (also with verification of binding to specific monoclonal antibodies) that recombinant protein was expressed in the host cell.

Example 10 Expression of T16 gene as a biomarker in breast cancer

The presence and over expression of T16 transcript in breast carcinoma is consistent with the differential cDNA cloning strategy which suggest its utility as a biomarker in breast cancer detection.

25 patients suspected of having cancer were tested for their T16 transcript by using RT-PCR in their peripheral blood as described in the experiment.

All 25 patients underwent biopsy and their condition assessed independently by the pathology department of Rabin Medical Center, Israel. The results are shown in Table 2, wherein (-) = no product, (±) = very faint band, (+) or (++) = strong bands.

As can be seen, of the 13 cancer patients 12 were identified by over expression of T16 with a single false-negative result (Case 12) which was clinically assessed as having cancer and not diagnosed by T16 over expression. These results indicate a false-negative level of about 9%. Of the 15 patients positively identified by the T16 transcript, 3 were not clinically diagnosed as having cancer, i.e. three false- positive results, indicating a 20% level of false-positive results.

Table 2

PCR Amplification of T16 transcripts in blood of patients with suspected breast cancer

No.	RTPCR 17-SPR*	Diagnosis of breast biopsy	Remarks
1	++	Inf. Duct Ca T 1.6 No/12	Cancer
2	-	Fibrocystic Disease	
3	-	Fibrocystic Disease	
4	+	Inf. Duct Ca	Cancer
5	-	Fibrocystic Disease	
6	-	Fibrocystic Disease	
7	++	Inf. Duct Ca GII T1.4 No/11	Cancer
8	++	Lobular Ca In-Situ	
9	-	Fibrocystic Disease	
10	-	Atypical hyperplasia	
11	+	Inf. Duct Ca GIII T1,2 N?	Cancer
12	-	Inf. Duct Ca GII T2 No/8	Cancer
13	+	Atypical hyperplasia + Fibrocystic Disease	
14	+	Inf. bular Ca. T1.8 N1/10	Cancer
15	+-	Large lactiferous ducts. Periductal lymphoid Inf.	
16	++	Inf. Duct Ca T2 No/15	Cancer
17	+	Inf. Duct Ca T2 No/8	Cancer
18	+	Fibrocystic Disease	
19	++	Inf. Duct Ca GII T2.5 N/9	Cancer
20	+	Inf. Duct Ca GIII T1 No/8	Cancer
21	-	Fibrocystic Disease Ductectasia	
22	+	Introductal papillary Ca T0.5	
23	++	Inf. Duct Ca GII T1 No/15	Cancer
24	-	Fibrocystic prol. Ductectasia Atypical hyperplasia	
24	+	Inf. Duct Ca GII-III T2 No/13	Cancer

5

* Amplification of T16 mRNA transcripts in blood of patients prior to breast biopsy

(-) No PCR product (+-) Very faint PCR product (+), (++) Positive PCR product

10

Example 11 The effect of OFF1 on granulocyte-monocyte propogation cells

OFF1 was purified from term placenta as previously described in U.S. 4,882,270 and U.S. 4,954,434 and will be termed hereinafter also as p43.

Mouse. MoAb. CM-H-9 was produced against human placental ferritin as previously described in the above two U.S. patents. The MoAb was obtained from ascites fluid following precipitation with 50% saturated ammonium sulphate solution, and purification on sephadex G-200 colomn.

CFU-GM ASSAYS

Bone marrow samples from 11 healthy volunteer donors were processed by density gradient separation using Histopaque-107 (Sigma diagnostics, St. Louis, MO, USA) to obtain a purified population of mononuclear cells. Colony assays were performed in a plating medium containing final concentrations of 0.92% methyl cellulose (M-281 powder, 4,000 centipoise, Fisher Scientific Co., Fair Lawn, NJ, USA), rehydrated in Iscove's modified Dulbecco's medium containing 36 mM sodium bicarbonate (Gibco, Grand Island, NY, USA), 30% fetal bovine serum (FBS) (HyClone, Logan, UT, USA)0.292 mg/ml glutamine, 100 U/ml penicillin and 0.01 mg/ml streptomycin (Biological Industries, Beit Haemek, Israel).

Growth factors used were 15-30 mg/ml GM-CSF Leucomax (Sandoz Pharma) and 5% vol/vol human phytohemagglutinin-M (Difco Laboratories, Detroit, MI, USA)-induced conditioned medium (Cond. Med.) p43 (PLF) was added at concentration of 1 μg/mL and in neutralization experiments, p43 (PLF) was preincubated with 10x excess of CM-H-9 MoAb at 37°C for 30 min and the complex added to the assay as above.

The colony assay medium contained 10^5 mononuclear cells/ml and each 1 ml was plated into triplicate wells (333 μl/well) of a 24 well tissue culture plate (Greiner, Germany). Water was added to spaces between cells to maximize humidity during incubation of the cultures. The cultures were incubated at 37°C in 5% CO_2 and 55% relative humidity. Plates were scored after 14 days for colonies containing more than 50 cells.

RESULTS

Effect of OFF1 protein (p43 (PLF)) on human CFU-GM growth in-vitro: Dose response

(p43 (PLF)) was tested for its capacity to influence colony formation of human bone marrow progenitor CFU-GM. p43 alone exhibited a concentration dependent stimulatory effect on bone marrow progenitor cells obtained from three donors. The highest number of colonies was obtained with 1μg/1mL of p43 (Fig. 10). At higher concentrations the number of cells was lower. All subsequent experiments were further carried out at concentration of 1 μg/mL of p43 (PLF).

Comparison of the stimulatory effect of p43 (PLF) and GM-CSF

The mean number of colonies obtained following treatment of bone marrow cells from 11 donors with p43 (PLF) was 169 +/- 216 significantly higher (p=0.028) than in medium only (18 +/- 21) (Table 3). The stimulatory effect of p43 (PLF) was abolished following preincubation with its specific MoAb CM-H9 Mean CFU-GM – 7 +/- 10) (Fig. 11). The number of colonies obtained with p43 (PLF) was not significantly different than the number obtained with Cond. Med. (227 +/- 223) or with GM-CSF (276 +/- 257) (Table 3). When the treatment combined both GM-CSF (15 ugr/mL) and p43 (PLF) the mean number of colonies obtained with the

mixture increased to 353 +/- 236 colonies, but it did not reach a statistical significance compared to each factor alone.

It is concluded that p43 (PLF) known to act as an immunosuppressive cytokine is active as a growth factor on human bone marrow progenitor cells.

Table 3

The effect of p43 (PLF) on the generation of CFU-GM formation by normal human bone marrow. Comparison with conditioned medium and with GMCSF.

Bone Marrow No.	None	TREATMENT		
		Cond.Med.	p43 (PLF) (1 μg/ml)	GM-CSF (15ng/ml)
1	8	522	280	144
2	46	758	748	396
3	46	322	152	114
4	2	90	28	66
5	2	134	94	136
6	8	170	6	250
7	0	52	0	50
8	4	102	242	74
9	0	54	8	520
10	34	170	210	884
11	48	126	94	405
AVG.	18	227	169	276.2
STD.	21	223	216	257
MEDIAN	8	134	94	144
RANGE	0-48	52-758	0-748	50-884

CLAIMS:

1. A DNA sequence coding for oncofetal ferritin 1 (OFF1) protein selected from the group consisting of:

(i) a DNA sequence as depicted in Fig. 1;

(ii) a DNA sequence as depicted in Fig. 4;

(iii) a DNA sequence which codes for the same amino acid sequences of (i) or (ii);

(iv) fragments of any of the sequences of (i) to (iii) that code for a physiologically active protein;

(v) a DNA sequence that has at least 80% homology, as determined by hybridization under stringent conditions, to any one of the sequences of (i) to (iv) and code for a physiologically active protein; and

(vi) a DNA sequence that hybridizes to the sequences of (i) or (iv), under highly stringent conditions, being hybridization to filter-bound DNA in 0.5M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C, which can either be used as a probe for OFF1, or which encodes functionally equivalent gene product; and

(vii) a DNA sequence that hybridizes to the sequences of (i) to (iv), or to the clone deposited with the ATCC, under moderately stringent conditions, e.g., washing in 0.2xSCC/0.1% SDS at 42°C yet which still encodes a functionally equivalent gene product.

2. An expression vector comprising the DNA sequence of Claim 1.

3. An expression vector according to Claim 2, being a plasmid.

4. A genetically engineered host cell containing the DNA sequence of Claim 1, operatively associated with a regulatory element heterologous to the DNA sequence which directs the expression of the DNA sequence by the host cell.

5. An amino acid sequence coded by the nucleic acid sequence of Claim 1.

6. A DNA sequence which is complementary to at least a portion of any one of the sequences of Claim 1, capable of being transcribed to mRNA which is an anti-sense to at least a portion of the mRNA transcribed by any one of the sequences of Claim 1, said portion being sufficient to inhibit translation of the mRNA to protein.

7. An anti-sense mRNA sequence transcribed from the DNA of Claim 6.

8. A pharmaceutical composition comprising the expression vector of Claim 3.

9. A pharmaceutical composition comprising the amino acid sequence of Claim 5.

10. A pharmaceutical composition according to Claims 8 or 9, for immunization against cancer.

11. A pharmaceutical composition according to Claim 10, for ~~immunization against breast cancer.~~

12. A pharmaceutical composition according to Claims 8 or 9, for the treatment of transplant rejections, autoimmune diseases, pathological pregnancies and for enhancing fertilization rates during IVF treatment.

13. A pharmaceutical composition according to Claims 8 or 9 for use as a growth factor of bone-marrow progenitor cells.

14. A pharmaceutical composition according to Claim 13, wherein the cells are granulocyte monocytes.

15. A growth factor for bone marrow progenitor cells comprising as an active ingredient the amino acid sequence of Claim 5.

16. An expression vector comprising the DNA of Claim 6.

17. A pharmaceutical composition comprising the expression vector of Claim 16.

18. A pharmaceutical composition comprising the anti-sense mRNA sequence of Claim 6.

19. A pharmaceutical composition according to Claim 17 or 18, for the treatment of cancer.

20. A pharmaceutical composition according to Claim 19 for the treatment of breast cancer.

21. A pharmaceutical composition according to Claim 17 or 18, for the induction of abortion.

22. A method for the diagnosis of cancer comprising: detecting elevated to levels of mRNA transcribed from DNA sequences depicted in Fig. 1 or Fig. 4.

23. A method according to Claim 22, wherein the cancer is selected from the group consisting of: breast cancer, hepatoblastoma, leukemia, Hodgkin's and non-Hodgkin's lymphomas and embryonal tumors.

24. A method for the detection of Downs' Syndrome, comprising detecting elevated levels of mRNA transcribed from the DNA sequence of Fig. 1 or 4.

25. A method for the detection of pathological pregnancies comprising detecting decreased levels of mRNA transcribed from the DNA sequence of Fig. 1 or 4.

26. A method according to Claim 25, wherein the pathological pregnancy is selected from the group consisting of: spontaneous abortion and miscarriage, premature contractions, toxemia, premature delivery.

27. A method according to any one of Claims 22 to 26, wherein the level of the DNA expression is detected using RT-PCR.

28. A method for isolating the DNA sequence of Fig. 1 or 4, substantially as hereinbefore described.

29. Primers for use in the methods of Claims 27 or 28 selected from the group consisting of:

5′ GGT GGC GAC GAC TCC TGG AGC CCG 3′
5′ TTG ACA CCA GAC CAA CTG GTA ATG 3′
5′ GAC CGC GAT GAT GTG GCT TTG AAG AAC 3′
5′ GAT AGG ATC TTT AGC GAC AGC CGA 3′
5′ ATG GCG GCC TCT GAG TCC TGG TGG 3′
5′ CGG GCT GAA TGC AAT GGA GTG TGC 3′
5′ GAC CCC CAT TTG TGT GAC 3′
5′ CGA CGA CTC CTG GAG CCC G 3′
5′ Biotin-TTG ACA CCA GAC CAA CTC GTA ATG 3′
5′ AGC CGA CAG CGA TTT CTA GGA TAG 3′
5′ GTT CTT CAA AGC CAC ATC ATC GCG GTC 3′
5′ GCT TTC ATT ATC ACT GTC TCC CAG GGT G 3′
5′ CAG ACG TTC TTC GCC GAG AGT CGT 3′
5′ CAG ACG TTC TTC GCC GAG AGT CGT CGG 3′
5′ CAT TTC GGG GAT TCG GGG GA 3′
5′ GGG GGA CGG AAC CCG GCG CT 3′
5′ CCC TCT ACA CTT ATC ATC TTC 3′
5′ CTA TCC TAG AAA TCG CTG TCG GCT 3′
5′ GTC ACT ACT GGA ATT CCC TTC TCC 3′
5′ GGA GAA GGG AAT TCC AGT AGT GAC 3′
5′ GGA AAT CGC TGT CGC CTA ACC 3′
5′ GGT TAG GCG ACA GCG ATT TCC 3′
5′ GGC CAC GCG TCG ACT AGT AC 3′
5′ GTA ATG CAC ACTCCA TTG GC 3′
5′ GTA ATG CAC ACT CCA TTG 3′
5′ GCG CTC AGC TGG AAT TCC 3′
5′ GGA ATT CCA GCT GAG CGC 3′
5′ GTG GGA TCC CCA TGA CGA CCG CGT CCA CC 3′
5′ GAC TCG AGT TAA GCC GAC AGC GAT TTC 3′
5′ GAC TCG AGT CAG GGT GAC CGA AAA ATC AG 3′
5′ CCC GCT CGA GTC AGG GTG ACC GAA AAA TCA G 3′

For the Applicants,
REINHOLD COHN AND PARTNERS
By:

110699-6-SPC-(00001)-TG/be/jg-10/09/98

5"

TTGACACCAG	ACCAACTGGT	AATGGTAGCG	ACCGGCGCTC	AGCTGGGATT	CCTAAAATG
TAATGCACAC	TCCATTGGCAT	TCAGCCCGCC	TCTCCTTAGT	CGCCGCC[illegible]	ACGACCGCGT
CCACCTCGCA	GGTGCGCCAG	AACTACCACC	AGGACTCAGA	GGCCGCCATC	AACCGCCAGA
TCAACCTGGA	GCTCTACGCC	TCCTACGTTT	ACCTGTCCAT	GTCTTACTAC	TTTGACCGCG
ATGATGTGGC	TTTGAAGAAC	TTTGCCAAAT	ACTTTCTTCA	CCAATCTCAT	GAGGAGAGGG
AACATGCTGA	GAAACTGATG	AAGCTGCAGA	ACCAACGAGG	TGGCCGAATC	TTCCTTCAGG
ATATCAAGAA	ACCAGACTGT	GATGACTGGG	AGAGCGGGCT	GAATGCAATG	GAGTGTGCAT
TACATTTGGA	AAAAAATGTG	AATCAGTCAC	TACTGGAATT	CCCTTCTCCT	ATCTCTCCCA
GTCCTAGCTG	CTGGCATCAC	TATACTACTA	ACAGACCGCA	ACCTCAACAC	CACCTTCTTC
GACCCCGCCG	GAGGAAGAGA	CCCCATTCTA	TACCAACACC	TATTCTGATT	TTTCGGTCAC
CC[illegible]AGTTT	ATATTCTTAT	CCTACCAGGC	TTCCGAATAA	TCTCCCATAT	TGTAACTTAC
TACTCCGGAA	ATCGCTGTCG	CCTAACCGCT	AACATTACTG	CAGGCCACCT	ACTCATGCAC
CTAATTGGAA	GCGCCACCCT	AGCAATATCA	ACCATTAACC	TTCCCTCTAC	ACTTATCATC
TTCACAATTC	TAATTCTACT	GACTATCCTA	GAAATCGCTG	TCGCCTTAAT	CCAAGCCTAC
GTTTTCACAC	TTCTAGTAA	GCCTCTACCT	GCACGACAAC	ACATAAAAAA	AA 3"

Fig. 1 Nucleic acid sequence of clone T16 isolated from T47D breast cancer cDNA library Initiation and termination codons of ORF are indicated.

Fig 1

CLONE #47 GGGCGACGGAACCCGG

CGCTCGTTCCCCACCCCGGCCGGCCGCCCATAGCCAGCCCTCCGTCAC

CLONE T 16 TTGACACCAG

CTCTTCACCGCACCCTCGGACTGCCCCAAGGCCCCCGCCGCCCCTCC
ACACCAACTGGTAATGGTAGCGACCGGCGCTCAGCTGGAATTCCAAAA

AGCGCCGCGCAGCCACCGCCGCCGCCGCCGCCTCTCCTTAGTCGCCGCC
AATGTAATCCACACTCCATTGCATTCAGCCCGCCTCTCCTTAGTCGCCGCC

ATG ACG ACC GCG TCC ACC TCG CAG GTG CGC CAG
ATG ACG ACC GCG TCC ACC TCG CAG GTG CGC CAG

AAC TAC CAC CAG GAC TCA GAG GCC GCC ATC AAC
AAC TAC CAC CAG GAC TCA GAG GCC GCC ATC AAC

CGC CAG ATC AAC CTG GAG CTC TAC GCC TCC TAC
CGC CAG ATC AAC CTG GAG CTC TAC GCC TCC TAC

GTT TAC CTG TCC ATG TCT TAC TAC TTT GAC CGC
GTT TAC CTG TCC ATG TCT TAC TAC TTT GAC CGC

GAT GAT GTG GCT TTG AAG AAC TTT GCC AAA TAC
GAT GAT GTG GCT TTG AAG AAC TTT GCC AAA TAC

TTT CTT CAC CAA TCT CAT GAG GAG AGG GAA CAT
TTT CTT CAC CAA TCT CAT GAG GAG AGG GAA CAT

GCT GAG AAA CTG ATG AAG CTG CAG AAC CAA CGA
GCT GAG AAA CTG ATG AAG CTG CAG AAC CAA CGA

GGT GGC CGA ATC TTC CTT CAG GAT ATC AAG AAA
GGT GGC CGA ATC TTC CTT CAG GAT ATC AAG AAA

CCA GAC TGT GAT GAC TGG GAG AGC GGG CTG AAT
CCA GAC TGT GAT GAC TGG GAG AGC GGG CTG AAT

GCA ATG GAG TGT GCA TTA CAT TTG GAA AAA AAT
GCA ATG GAG TGT GCA TTA CAT TTG GAA AAA AAT

GTG AAT CAG TCA CTA CTG GAA CTG CAC AAA CTG
GTG AAT CAG TCA CTA CTG GAA TTC CCT TCT CCT

GCC ACT GAC AAA AAT GAC CCC CAT TTG TGT GAC
ATC TCT CCC AGT CCT AGC TGC TGG CAT CAC TAT

TTC ATT GAG ACA CAT TAC CTG AAT GAG CAG GTG
ACT ACT AAC AGA CGG CAA CCT CAA CAC CAC CTT

AAA GCC ATC AAA GAA TTG GGT GAC CAC GTG ACC
CTT CGA CCC CGC CGG AGG AAG AGA CCC CAT TCT

AAC TTG CGC AAG ATG GGA GCG CCC GAA TCT GGC
ATA CCA ACA CCT ATT CTG ATT TTT CGG TCA CCC

TTG GCG GAA TAT CTC TTT GAC AAG CAC ACC CTG
TGA AGTTTATATTCTTATCCTACCAGGCTTCGGAATAATCTCCCATATT

GGA GAC AGT GAT AAT GAA AGC TAA GCCTCGGGCTAATT
GTAACTTACTACTCCGGAAATCGCTGTCGCCTAACCGCTAACATTACTGC

TCCCATAGCCGTGGGGTGACTTCCCTGGTCACCAAGGCAGTGCATGCAT
AGGCCACCTACTCATGCACCTAATTGGAAGCGCCACCCTAGCAATATCA

GCATGTTGGGGTTTCCTTTACCTTTTCTATAAGTTGTACCAAAACATCCAC
ACCATTAACCTTCCCTCTACACTTATCATCTTCACAATTCTAATTCTACTG

TTAAGTTCTTTGATTTGTACCATTCCTTCAAATAAAGAAATTTGGTACCCA
ACTATCCTAGAAATCGCTGTCGCCTTAATCCAAGCCTACGTTTTCACACT

AAAAAAAA
TCTAGTAAGCCTCTACCTGCACGACAAAACATAAAAA

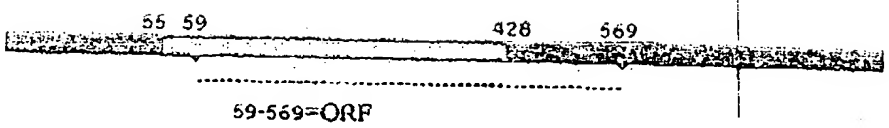


Fig. 2B Scheme of nucleic acid homology between cDNA clones T16 and p4,7 derived from T47D breast cancer cells and human placenta respectively. The shaded area represents different nucleic acid sequence. Initiation and termination of ORF.

Alignment

T16 p43 M459-920 to Hummtcg

```
ypa
5 type p43rev.comparetomito

 DESTFIT of: P43rev.Seq  check: 2305  from: 459  to: 920

LOCUS       P43REV      1104 BP DS-DNA              ENTERED   04/07/94
DEFINITION  -
ACCESSION   -
KEYWORDS    -
SOURCE      -
  ORGANISM  - . . .

 to: Hummtcg.Gb_Pr  check: 9132  from: 1  to: 16569

LOCUS       HUMMTCG     16569 bp ds-DNA   Circular  PRI       19-APR-1991
DEFINITION  Human mitochondrion, complete genome.
ACCESSION   J01415 M12648 M58503 M63932 M63933
KEYWORDS    12S ribosomal RNA; 16S ribosomal RNA; ATPase; circular;
            complete genome; cytochrome; cytochrome oxidase; displacement loop;
            oxidase; ribosomal RNA; transfer RNA; transfer RNA-Ala; . . .

 Symbol comparison table: Genecoredisk:[Gcgcore.Data.Rundata]Swgapdna.Cmp
 CompCheck: 5230

        Gap Weight:  3.000      Average Match:  1.000
     Length Weight:  0.300   Average Mismatch: -0.900

            Quality:  307.1              Length:    209
              Ratio:  0.991                Gaps:      0
 Percent Similarity: 99.522   Percent Identity: 99.522

 P43rev.Seq x Hummtcg.Gb_Pr May 20, 1994  09:00  . .

     463 CTTCTCCTATCTCTCCCAGTCCTAGCTGCTGGCATCACTATACTACTAAC 512
         ||||||||||||||||||||||||||||||||||||||||||||||||||
    6486 CTTCTCCTATCTCTCCCAGTCCTAGCTGCTGGCATCACTATACTACTAAC 6535

     513 AGACCGCAACCTCAACACCACCTTCTTCGACCCCGCCGGAGGAAGAGACC 562
         ||||||||||||||||||||||||||||||||||||||||||||||||||
    6536 AGACCGCAACCTCAACACCACCTTCTTCGACCCCGCCGGAGGAGGAGACC 6585

     563 CCATTCTATACCAACACCTATTCTGATTTTTCGGTCACCCTGAAGTTTAT 612
         ||||||||||||||||||||||||||||||||||||||||||||||||||
    6586 CCATTCTATACCAACACCTATTCTGATTTTTCGGTCACCCTGAAGTTTAT 6635

     613 ATTCTTATCCTACCAGGCTTCGGAATAATCTCCCATATTGTAACTTACTA 662
         ||||||||||||||||||||||||||||||||||||||||||||||||||
    6636 ATTCTTATCCTACCAGGCTTCGGAATAATCTCCCATATTGTAACTTACTA 6685

     663 CTCCGGAAA 671
         |||||||||
    6686 CTCCGGAAA 6694
```

Fig. 3 Sequence Homology between cDNA clone T16 and human mitochondrial DNA.

TTGACACCAG	ACCAACTGGT	AATGGTAGCG	ACCGGCGCTC	AGCTGGGATT	CCTAAAATGT
TAATGCACAC AATGCACACT	TCCATTGCAT CCATTGGCAT	TCAGCCCGCC	TCTCCTTAGT	CGCCGCC[redacted]	ACGACCGCGT
CCACCTCGCA	GGTGCCCCAG	AACTACCACC	AGGACTCAGA	GGCCGCCATC	AACCGCCAGA
TCAACCTGGA	GCTCTACGCC	TCCTACGTTT	ACCTGTCCAT	GTCTTACTAC	TTTGACCGCG
ATGATGTGGC	TTTGAAGAAC	TTTGCCAAAT	ACTTTCTTCA	CCAATCTCAT	GAGGAGAGGG
AACATGCTGA	GAAACTGATG	AAGCTGCAGA	ACCAACGAGG	TGGCCGAATC	TTCCTTCAGG
ATATCAAGAA	ACCAGACTGT	GATCACTGGG	AGAGCGGGCT	GAATGCAATG	GAGTGTGCAT
TACATTTGGA	AAAAAATGTG	AATCAGTCAC	TACTGGAATT	CCCTTCTCCT	ATCTCTCCCA
GTCCTAGCTG	CTGGCATCAC	TATACTACTA	ACAGACCGCA	ACCTCAAGAC	CACCTTCTTC
GACCCCGCCG	GAGGAAGAGA	CCCCATTCTA	TACCAACACC	TATTCTGATT	TTTCGGTCAC
CC[redacted]AGTTT	ATATTCTTAT	CCTACCAGGC	TTCGGAATAA	TCTCCCATAT	TGTAACTTAC
TACTCCGGAA	ATCGCTGTCG	CCTAACCGCT	ATCATTACTG	CAGGCCACCT	ACTCATGCAC
CTAATTGGAA	GCGCCACCCT	AGCAATATCA	ACCATTAACC	TTCCCTCTAC	ACTTATCATC
TTCACAATTC	TAATTCTACT	GACTATCCTA	GAAATCGCTG	TCGCCTTAAT	CCAAGCCTAC
GTTTTCACAC	TTCTAGTAA	GCCTCTACCT	GCACGACAAC	ACATAAAAAA	AA

Fig. 4 Comparison of n.a. sequence between placental cDNA obtained by PCR amplification using T16 specific primers (upper clone) and T16 cDNA T16 cDNA clone (lower) identical n.a.

FIG 4

AATGCACAGTCCATTGCATTCAGCCCGCCTCTCCTTAGTCGCCGCC

met	thr	thr	ala	ser	thr	ser	gln	val	arg	gln					
ATG	ACG	ACC	GCG	TCC	ACC	TCG	CAG	GTG	CGC	CAG					
asn	tyr	his	gln	asp	ser	glu	ala	ala	ile	asn					
AAC	TAC	CAC	CAG	GAC	TCA	GAG	GCC	GCC	ATC	AAC					
arg	gln	ile	asn	leu	glu	leu	tyr	ala	ser	tyr					
CGC	CAG	ATC	AAC	CTG	GAG	CTC	TAC	GCC	TCC	TAC					
val	tyr	leu	ser	met	ser	tyr	tyr	phe	asp	arg					
GTT	TAC	CTG	TCC	ATG	TCT	TAC	TAC	TTT	GAC	CGC					
asp	asp	val	ala	leu	lys	asn	phe	ala	lys	tyr					
GAT	GAT	GTG	GCT	TTG	AAG	AAC	TTT	GCC	AAA	TAC					
phe	leu	his	gln	ser	his	glu	glu	arg	gln	his					
TTT	CTT	CAC	CAA	TCT	CAT	GAG	GAG	AGG	GAA	CAT					
ala	glu	lys	leu	met	lys	leu	gln	asn	gln	arg					
GCT	GAG	AAA	CTG	ATG	AAG	CTG	CAG	AAC	CAA	CGA					
gly	gly	arg	ile	phe	leu	gln	asp	ile	lys	lys					
GGT	GGC	CGA	ATC	TTC	CTT	CAG	GAT	ATC	AAG	AAA					
pro	asp	cys	asp	asp	trp	glu	ser	gly	leu	asn					
CCA	GAC	TGT	GAT	GAC	TGG	GAG	AGC	GGG	CTG	AAT					
ala	met	glu	cys	ala	leu	his	leu	glu	lys	asn					
GCA	ATG	GAG	TGT	GCA	TTA	CAT	TTG	GAA	AAA	AAT					
val	asn	gln	ser	leu	leu	glu	phe	pro	ser	pro					
GTG	AAT	CAG	TCA	CTA	CTG	GAA	TTC	CCT	TCT	CCT					
ile	ser	pro	ser	pro	ser	cys	trp	his	his	thr					
ATC	TCT	CCC	AGT	CCT	AGC	TGC	TGG	CAT	CAC	TAT					
thr	thr	asn	arg	pro	glu	pro	gln	his	his	leu					
ACT	ACT	AAC	AGA	CCG	GAA	CCT	CAA	CAC	CAC	CTG					
leu	arg	pro	arg	arg	arg	lys	arg	pro	his	ser					
CTT	CGA	CCC	CGC	CGG	CGG	AAG	AGA	CCC	CAT	TCT					
ile	pro	thr	pro	ile	leu	ile	phe	arg	ser	pro					
ATA	CCA	ACA	CCT	ATT	CTG	ATT	TTT	CGG	TCA	CCC					

TCGAAGTTTATATTCTTATCCTACCAGGCTTCGGAATAATCTCCCATATTGTAACTTAC

TACTCCGGAAATCGCTGTCGCCTAACCGCTAACATTACTGCAGGCCACCTACTCATGCAC

CTAATTGGAAGCGCCACCCTAGCAATATCAACCATTAACCTTCCCTCTACACTTATCATC

TTCACAATTCTAATTCTACTGACTATCCTAGAAATCGCTGTCGCCTTAATCCAAGCCTAC

GTTTTCACACTTTGGTACCCAAAAAAAA

Fig. 5 Nucleic acid sequence and deduced amino acid sequence of T16 cDNA.

Fig 5

patent

	HEART	BRAIN	PLACEN	LUNG	LIVER	SK. MUSC	KIDNEY	PANCREAS
S30/ACTIN	220	148	116	187	287	155	230	260
SPF-16R/ACTIN	195	115	70	60	281	127	260	170

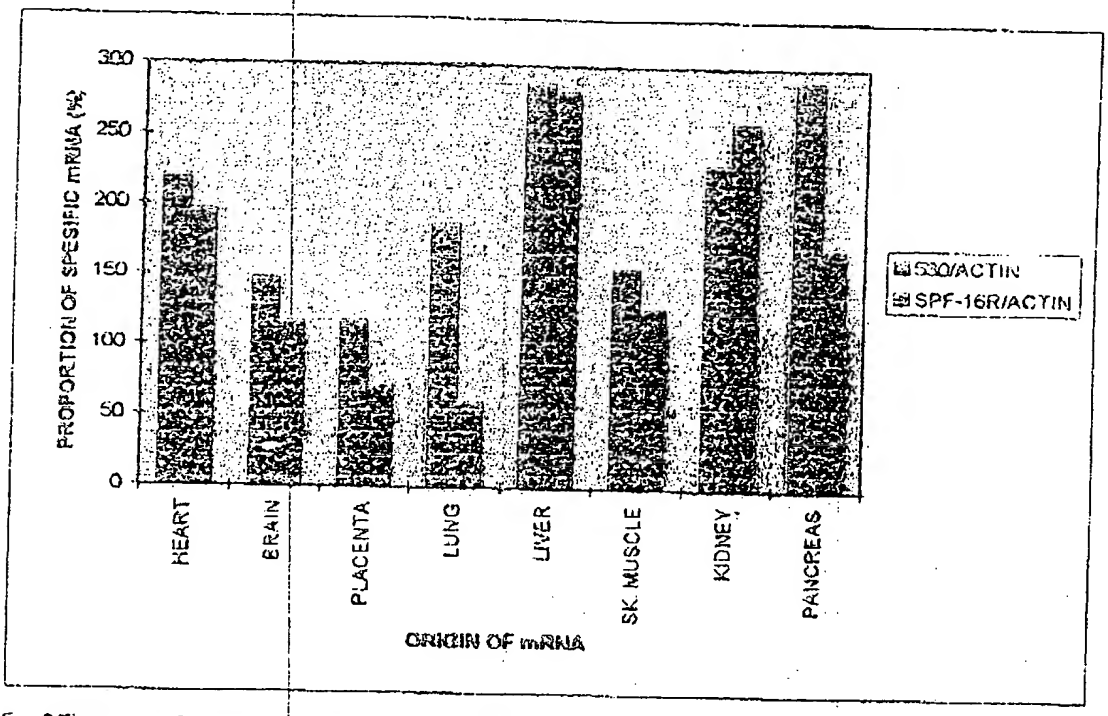


Fig. 6 Tissue specific mRNA expression analysis. Relative expression of FTH mRNA hybridized with 32P Pst1 3' fragment of liver FTH cDNA (S30 bp) and T16 specific mRNA hybridized with T16 fragment (161 n.a.) obtained by PCR using primers SPF-16R. A- RNA from normal human adult tissuses (Clontec cat. # 7760-1).

Fig 6A

Fig 6A

RATIO OF ACTIN (PSL)	HBL	T47D	MCF7
330/ACTIN	58.2	77.8	89
SPF-16R/ACTIN	35.4	150.4	134.7

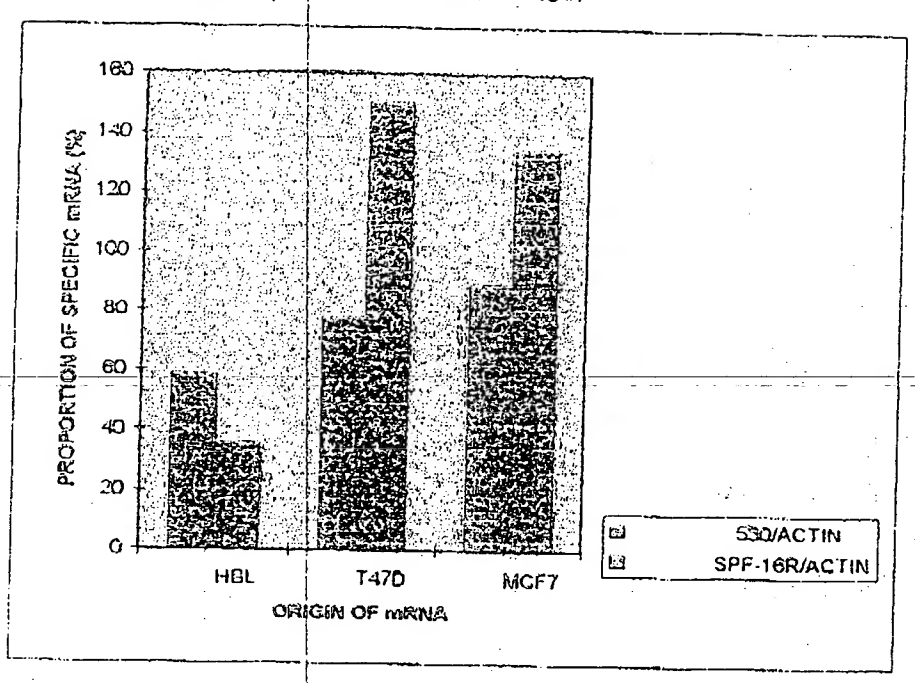


Fig. 6 Tissue specific mRNA expression analysis. Relative expression of FTH mRNA hybridized with 32P Pst1 3' fragment of liver FTH cDNA (330 bp) and T16 specific mRNA hybridized with T16 fragment (161 n.a.) obtained by PCR using primers SPF-16R.

B Human breast cancer cell line (MCF-7 and T47D) and lactating breast epithelial cell line (HBL-100).

Fig 6B

TTGACACCAG	ACCAACTGGT	AATGGTAGCG	ACCGGCGCTC	AGCTGGAATT	CCAAAAAATC
TAATGCACAC	TCCATTGCAT	TCAGCCCGCC	TCTCCTTAGT	CGCCGCC[redacted]	ACGACCGCGT
CCACCTCGCA	GGTGCGCCAG	AACTACCACC	AGGACTCAGA	GGCCGCCATC	AACCGCCAGA
TCAACCTGGA	GCTCTACGCC	TCCTACGTTT	ACCTGTCCAT	GTCTTACTAC	TTTGACCGCG
ATGATGTGGC	TTTGAAGAAC	TTTGCCAAAT	ACTTTCTTCA	CCAATCTCAT	GAGGAGAGGC
AACATGCTGA	GAAACTGATG	AAGCTGCAGA	ACCAACGAGG	TGGCCGAATC	TTCCTTCAGG
ATATCAAGAA	ACCAGACTGT	GATGACTGGG	AGAGCGGGCT	GAATGCAATG	GAGTGTGCAT
TACATTTGGA	AAAAAATGTG	AATCAGTCAC	TACTGGAATT	CCCTTCTCCT	ATCTCTCCCA
GTCCTAGCTG	CTGGCATCAC	TATACTACTA	ACAGACCGCA	ACCTCAACAC	CACCTTGTTC
GACCCCGCCG	GAGGAAGAGA	CCCCATTCTA	TACCAACACC	TATTCTGATT	TTTCGGTCAC
CC[redacted]AGTTT	ATATTCTTAT	CCTACCAGGC	TTCGGAATAA	TCTCCCATAT	TGTAACTTAC
TACTCCGGAA	ATCGCTGTCG	CCTAACCGCT	AACATTACTG	CAGGCCACCT	ACTCATGCAC
CTAATTGGAA	GCGCCACCCT	AGCAATATCA	ACCATTAACC	TTCCCTCTAC	ACTTATCATC
TTCACAATTC	TAATTCTACT	GACTATCCTA	GAAATCGCTG	TCGCCTTAAT	CCAAGCCTAC
GTTTTCACAC	TTCTAGTAA	GCCTCTACCT	GCACGACAAC	ACATAAAAAA	AA

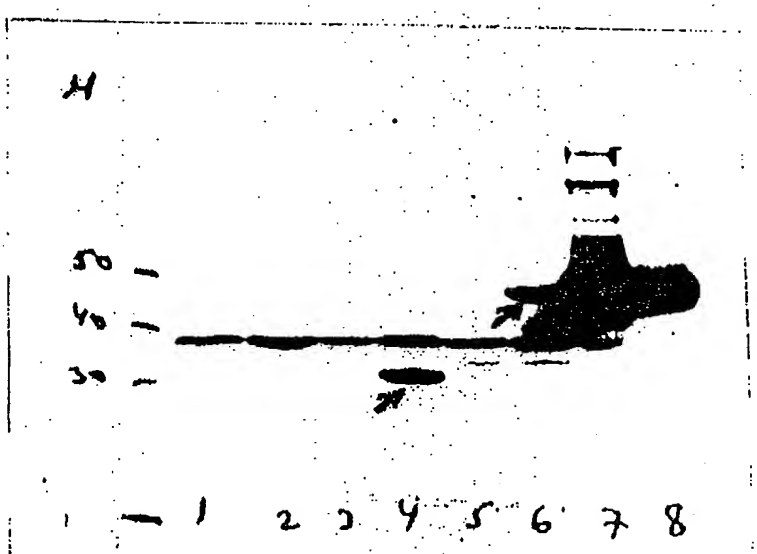
Fig. 7 Shaded areas represent the localization of the sequences used as primers.

Fig 7

TTGACACCAG	ACCAACTGGT	AATGGTAGCG	ACCGGCGCTC	AGCTGGAATT	CCAAAAAATG
TAATGCACAC	TCCATTGCAT	TCAGCCCGCC	TCTCCTTAGT	CGCCGCC[redacted]	ACGACCGCGT
CCACCTCGCA	GGTGCGCCAG	AACTACCACC	AGGACTCAGA	GGCCGCCATC	AACCGCCAGA
TCAACCTGGA	GCTCTACGCC	TCCTACGTTT	ACCTGTCCAT	GTCTTACTAC	TTTGACCGCG
ATGATGTGGC	TTTGAAGAAC	TTTGCCAAAT	ACTTTCTTCA	CCAATCTCAT	GAGGAGAGGG
AACATGCTGA	GAAACTGATG	AAC[redacted] PstI	ACCAACGAGG	TGGCCGAATC	TTCCTTCAGG
ATATCAAGAA	ACCAGACTGT	GATGACTGGG	AGAGCGGGCT	GAATGCAATG	GAGTGTGCAT
TACATTTGGA	AAAAAATGTG	AATCAGTCAC	TAC[redacted] ECOR1 T	CCCTTCTCCT	ATCTCTCCCA
GTCCTAGCTG	CTGGCATCAC	TATACTACTA	ACAGACCGCA	ACCTCAACAC	CACCTTCTTC
GACCCCGCCG	GAGGAAGAGA	CCCCATTCTA	TACCAACACC	TATTCTGATT	TTTCGGTCAC
CC[redacted]AGTTT	ATATTCTTAT	CCTACCAGGC	TTCGGAATAA	TCTCCCATAT	TGTAACTTAC
TACTCCGGAA	ATCGCTGTCG	CCTAACCGCT	AACATTACTG	CAGGCCACCT	ACTCATGCAC
CTAATTGGAA	CCGCCACCCT	AGCAATATCA	ACCATTAACC	TTCCCTCTAC	ACTTATCATC
TTCACAATTC	TAATTCTACT	GACTATCCTA	GAAATCGCTG	TCGCCTTAAT	CCAAGCCTAC
GTTTTCACAC	TTCTAGTAA	GCCTCTACCT	GCACGACAAC	ACATAAAAAA	AA

Restriction Enzyme Map.

Fig 8



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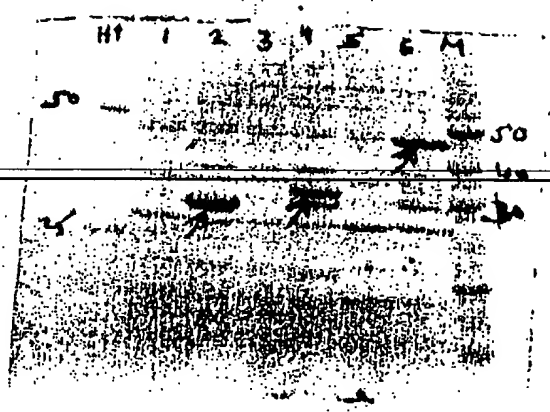

A

Fig 1. Expression of GST fusion proteins of OFF1 and its C48 fragment in E coli

A. SDS-PAGE (12%) of total cell lysates 10μl. track 2: from E.coli containing the vector pGEX alone or the pGEX constra
containing the C48 fragment track 4, or total construct contain

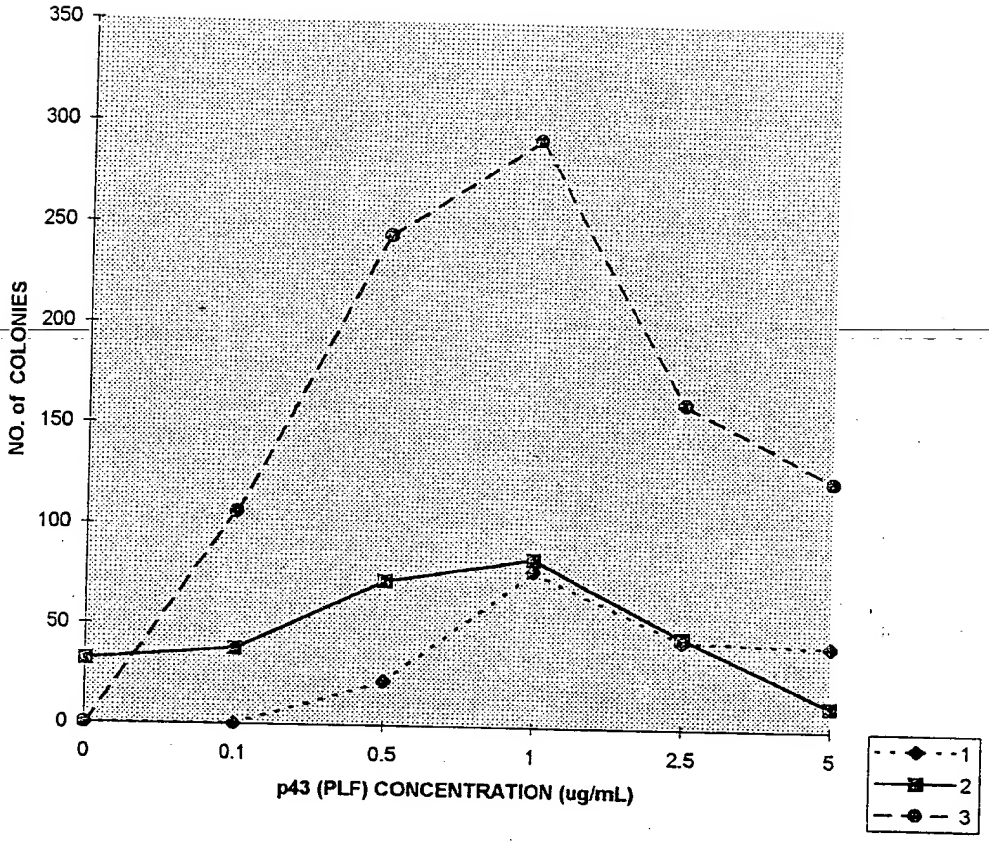

Fig.1 Effect of p43(PLF) on CFU-GM colony formation. Dose response of bone-marrow cells obtained from 3 healthy donors.

Fig 10

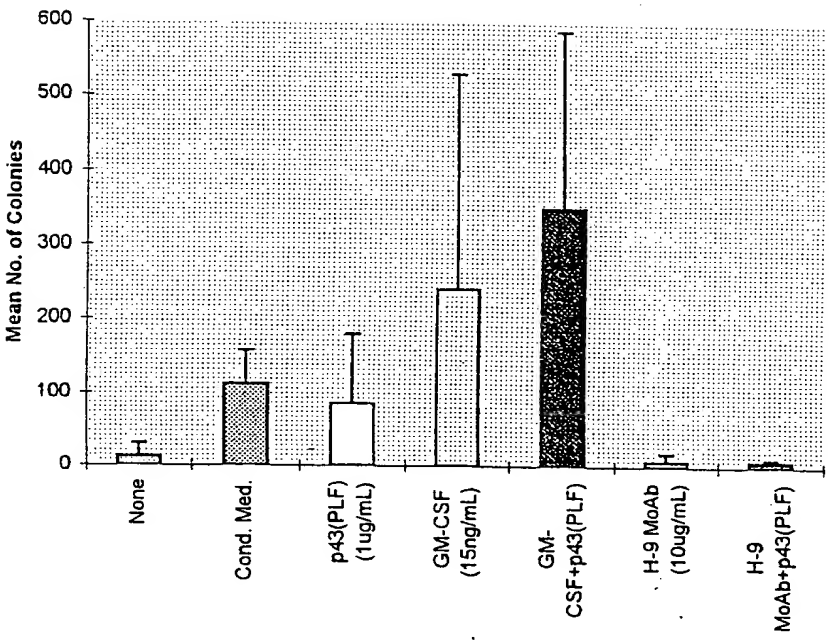

Fig. 2: The effect of p43 (PLF) and its combination with GM-CSF or CM-H-9 MoAb on CFU-GM colony formation.

Fig 11